

Test for Ready Biodegradability of Products.

- 1 Sample designation: **"Bio Clean"**
- 1.1 Sample ID: 97TE144841
- 1.2 Sample characterization: amber liquid with characteristic odour
- 1.3 Sample received on: november 6th,1997
- 1.4 Storage conditions: Room temperature

- 2 Sponsor: Shieer Alpha GmbH
- 2.1 Address: Am Dorfplatz 14, D-23826 Todesfelde

- 3 Testing facility: Institut Fresenius GmbH, Taunusstein, Sect. Environmental Biology
- 3.1 Address: Im Maisel 14, D-65232 Taunusstein-Neuhof; Germany
- 3.2 Study director: Dr. H. Lebertz

- 4 Test method: OECD-Guideline 301 B (CO₂ Evolution Test)
- 4.1 Inoculum: Filtrate of homogenated activated sludge from the waste-water treatment plant at Taunusstein-Bleidenstadt; Lot-No.: 11.02.98
- 4.2 Application: 28 days
- 4.3 Detection method: CO₂-measurement

- 5 Control: Sodiumbenzoate, ≈20 mg/L TOC

- 6 Definitions:

TOC: total organic carbon

TCO₂: theoretical amount of CO₂ which may be developed from the test substance (expressed as mg CO₂ / g test substance). This value is calculated from the carbon content of the test substance and the relation of molar masses of CO₂ (= 44,01) und carbon (= 12,01).

ThCO₂: theoretical amount of CO₂, which may be developed from the test substance within the whole test solution (= 3.5 L).

- 7 Method description:

The test substance and the polyvalent inoculum from an activated sewage plant dealing predominantly with domestic sewage are incubated together in a mineral nutrient medium at 19-25 °C. The test substance is the sole carbon and energy source. The test solutions are aerated with CO₂-free compressed air and are stirred on a magnetic stirrer. When the test material is mineralized it is converted to CO₂ which is trapped in a system of gas-washing bottles into Bariumhydroxide. The CO₂ is quantified by titration of the remaining Bariumhydroxide with HCl. Comparing the amount of CO₂ produced upon degradation of the test substance with the theoretical amount of CO₂ (ThCO₂) the percentage of degradation is calculated. Two Blank controls with inoculum but without any test substance is run in

parallel in order to determine the amount of CO₂ derived from the inoculum. The mean of these values have to be subtracted from those values determined for the test solutions with the test substance. At the end of the test the reaction in the test solutions is stopped by addition of 1 mL HCl conc. to each of the test solutions, and inorganic carbonates are made volatile. Aeration with CO₂ -free compressed air is continued for another 1 day (or 2 days) in order to purge the remaining CO₂ off the test solutions. Two test solutions with the test substance are tested in parallel at 10 to 20 mg/L TOC. The test duration normally is 28 days (+ 1 day purging off the dissolved CO₂ from the test solutions after acidification of the test solutions).

8 Evaluation

$$\% \text{ TCO}_2 = \frac{\text{mg CO}_2 \text{ produced} \times 100}{(\text{mg Testsubstance in the test solution}) \times (\text{TCO}_2)} = \% \text{ Degradation}$$

9 Test report

The test solutions were prepared according to the OECD guideline 301 B.

Carbon content of the Test
substance (calculated from
TOC-measurement):

11.2 mg C / g Test substance

Relation molar masses of CO₂/C: 44.01 : 12.01 = 3.667

Resulting: TCO₂ = 41.042 mg CO₂ / g Test substance

Volume of test solutions: 3500 mL

Amount of test substance within:

Test solution 1: 3109 mg / 3.5 L

ThCO₂ = 128 mg CO₂ / 3500 mL Test solution

Test Solution 2: 3107 mg / 3.5 L

ThCO₂ = 128 mg CO₂ / 3500 mL Test solution

Amount of control within:

Test Solution 3 (with ≈ 20 mg/L TOC): 120 mg / 3.5 L Test solution

ThCO₂ = 258 mg CO₂ / 3500 mL Test solution

10 Results

The results are given in table 1 and 2, and a plot of the degradation curve is included in the report.

- 10.1 Total-CO₂-Evolution of the Blank: 94.8 mg CO₂ / 3.5 L in 28 d (+ 1d purging off the dissolved CO₂ from the test solutions after acidification of the test solutions)
- 10.2 Control: The control was degraded 86 % within 28 days. The threshold of "ready biodegradability" of ≥ 60 % was passed within 5 days.
- 10.3 Test substance: The threshold of "ready biodegradability" of ≥ 60 % was met within 28d in both test solutions. The "10-days-window" is not taken into consideration as the test substance is a mixture of different components. The final mean degradation value was 67%. The test substance "**Bio Clean**" may be termed "readily biodegradable" as given by the OECD Guideline 301B.

INSTITUT FRESENIUS GmbH

- Environmental Biology -

D-65232 Taunusstein-Neuhof, date: 03-12-98

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**Results of the Study on the Biodegradability according to
 CO₂ Evolution-Test, OECD 301 B**

Test substance: "**Bio Clean**"

Data	Time	Concentration: 3109 mg / 3.5 L ThCO ₂ : 128 mg CO ₂ / 3.5 L	
		mg CO ₂ evolved from the test substance, cumulative	% TCO ₂ (= % Degradation)
02-13-98	2d	6.77	5
02-16-98	5d	26.55	21
02-18-98	7d	41.48	33
02-20-98	9d	51.27	40
02-23-98	12d	55.92	44
02-27-98	16d	59.97	47
03-04-98	21d	66.94	53
03-11-98	28d	79.03	62 ¹⁾
03-12-98	29d	84.95	67 ²⁾

Tabelle 1b: Test Solution 2

Data	Time	Concentration: 3107 mg / 3.5 L ThCO ₂ : 128 mg CO ₂ / 3.5 L	
		mg CO ₂ evolved from the test substance, cumulative	% TCO ₂ (= % Degradation)
02-13-98	2d	5.42	4
02-16-98	5d	22.82	18
02-18-98	7d	37.38	29
02-20-98	9d	48.74	38
02-23-98	12d	54.42	43
02-27-98	16d	57.17	45
03-04-98	21d	67.72	53
03-11-98	28d	79.23	62 ¹⁾
03-12-98	29d	84.87	67 ²⁾

¹⁾ The test was stopped by the addition of 1 mL of HCl conc. to the test solution.

²⁾ The difference between this value and that before is due to the acidification of the test substance.

"Bio Clean"
Biodegradability according to OECD 301 B
CO₂-Evolution Test

